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The effect of CO₂ on carbon isotope discrimination in C₄ monocots (grasses) and dicots

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The presence or prevalence of C₄ plants within paleoecosystems is widely invoked to gain insight into paleoclimate conditions, herbivore paleoecology, landscape evolution, and more. Stable carbon isotope analyses of fossilized terrestrial substrates, such as paleosols, are a preferred method for such studies, due to the conspicuously high carbon isotope value ($\delta^{13}\text{C}$) of C₄ plant tissue, relative to C₃ plants growing in the same environment. Because C₄ photosynthesis features the enzyme PEP-carboxylase, which works to actively pre-concentrate CO₂ prior to fixation by RuBP, workers have assumed the $\delta^{13}\text{C}$ of C₄ plants to be independent of the CO₂ level of the atmosphere. We set out to test this assumption across a wide range of CO₂ (400-1400 ppm) in a diverse set of C₄ plants. Specifically, we included three C₄ dicot genera (*Amaranthus*, *Atriplex* and *Gomphrena*), extant weedy species commonly found in North America. To compliment these experiments, we included four genera of monocots (*Eleusine*, *Dactyloctenium*, *Sorghum*, *Setaria*), all grasses. We found strongly divergent trends between the two groups: all monocots showed no change in $\delta^{13}\text{C}$ value with increasing CO₂. All dicots, however, showed strong linear decreases ($R^2 = 0.97\text{-}0.99$) in $\delta^{13}\text{C}$ value with increasing CO₂ level. These results suggest that when quantifying C₃ versus C₄ abundances using $\delta^{13}\text{C}$ data, changes in CO₂ level must be considered when estimating endmember $\delta^{13}\text{C}$ values, particularly when significant dicot contribution is obvious, or cannot be ruled out.